The S-Monovette® RNA Exact stabiliser solution maintains sample integrity over multiple freeze/thaw cycles without loss of quality

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Introduction

RNA molecules are considered highly unstable, especially since the sample can easily become contaminated with ubiquitous RNases. Even in living organisms, RNA can easily be destroyed by oxidative reactions, spontaneous hydrolysis and enzymes. It is therefore important to stabilise samples that are taken for subsequent RNA analysis against degradation.

Due to the instability of RNA molecules, it is common practice to freeze both stabilised and non-stabilised samples at -80°C. It is also customary to thaw the samples in order to take an aliquot for sample isolation and then re-freeze them. Even when samples have been gathered together for a test series, it is customary to freeze the samples for a short period and then thaw them for joint processing. However, this standard procedure runs up against the fact that repeated freeze/thaw cycles are detrimental to sample integrity and ought to be avoided. We have therefore investigated the performance of the S-Monovette® RNA Exact stabiliser solution in repeated freeze/thaw cycles.

Pre-analytics

Result

The purity of the samples subjected to multiple freeze/thaw cycles, was shown using OD 260/280 ratios, which is a measure of contamination with proteins. OD 260/280 ratios were comparable to the controls, at an average of 2.1. The mean of the photometrically determined concentration (yield) for the samples subjected to multiple freeze/thaw cycles was 72 ng/µl. The RNA concentration of the control samples was 18.6% higher on average. Nevertheless, the sample integrity (RIN) of the samples subjected to multiple freeze/thaw cycles is unchanged compared to the control samples, at an average of 8.8.

	RIN	STDEV
Thawed 4x	8.8	0.2
Control	8.8	0.29

The sample integrity was also checked using quantitative RT PCR and shown using so-called ΔΔ CT evaluation. Here, having taken into account isolation fluctuations above the average of the CT value of three housekeeping genes (Δ), the change in the samples subjected to multiple freeze/thaw cycles compared to the control samples is shown (ΔΔ). A value here that is as close to 0 as possible indicates that no changes have occurred in the initial concentration of this mRNA.

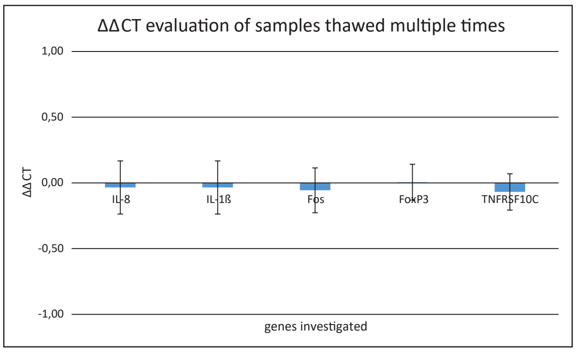

Figure:
Depiction of the conserved expression levels of genes after freeze-thaw cycles. RNA from S-Monovette® RNA Exact stabilized blood was either isolated directly after blood collection or after four freeze-thaw-cycles. cDNA was synthesized and expression levels of selected genes were compared to the levels obtained from the directly isolated sample with the ddCt-method.

Discussion

The experiment shows that repeatedly thawing and re-freezing samples stabilised with RNA Exact can result in a slight loss in yield. Thawing and re-freezing 4 times resulted in an average loss of 18.6% in the 8 tested donors.
The RNA integrity (RIN) and the ratios of the various mRNAs to one another (tested on 5 different genes) in samples stabilised with RNA Exact were not affected by the 4 freeze/thaw cycles performed.
The S-Monovette® RNA Exact stabiliser solution is able to ensure sample integrity over multiple freeze/thaw cycles without loss of quality. As it was already previously established that long-term storage of isolated RNA, even at -80°C, leads to a loss in quality, S-Monovette® RNA Exact now offers an alternative solution to sample storage, making it possible to process aliquots from the sample and re-freeze the rest, for example. This goes some way to making day-to-day work much easier.

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